

All-purpose protein sample QC using a novel micro-volume platform with cDrop software

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Introduction

Accurate total protein quantitation of purified proteins, cell lysates, affinity-column fractions or other complex protein samples is crucial for robust biochemical testing during biopharmaceutical development as well as production. As the presence of chemicals doesn't allow simple A280 protein quantification, more labor-intensive alternatives for quantification are used. Examples are the colorimetric Bradford or BCA assay, which negate to a large extent the presence of contaminants but require multiple steps and show variation depending on the sample composition and time point of measuring within the ongoing reaction.

A novel microfluidic method developed by Trinean allows direct total protein quantification compatible with complex samples. Using Trinean's proprietary cDrop software, a classic UV/VIS spectrum is analyzed to differentiate the protein content from interfering nucleic acids, co-factors or UV-absorbing buffer components, allowing quick, standardized total protein quantification.

Trinean's micro-volume platforms



DropSense

- Load 2 µl samples on a microfluidic slide.
- No evaporation or cross contamination.
- Simply insert the slide and select an application.
- 16 samples measured in approximately 2 minutes.



DropSense96

- Robotic or manual loading of 2 µl samples.
- Samples are preserved prior to read-out.
- Full 96-well DropPlate measured in 5 minutes.
- Software for robot integration or regulatory labs.

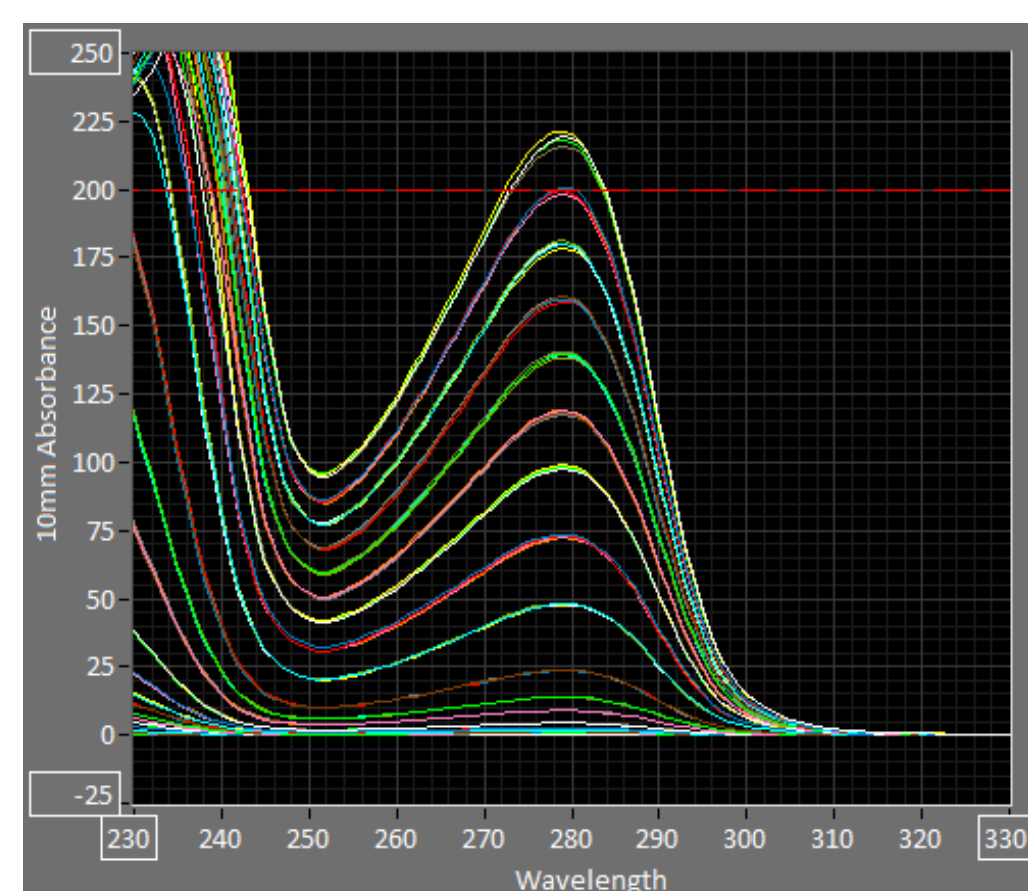
A280 protein quantification



Principle
Measured UV/VIS spectrum
↓
Protein concentration =
A280 of white curve * given E1%

1. Linear range of A280 protein quantification

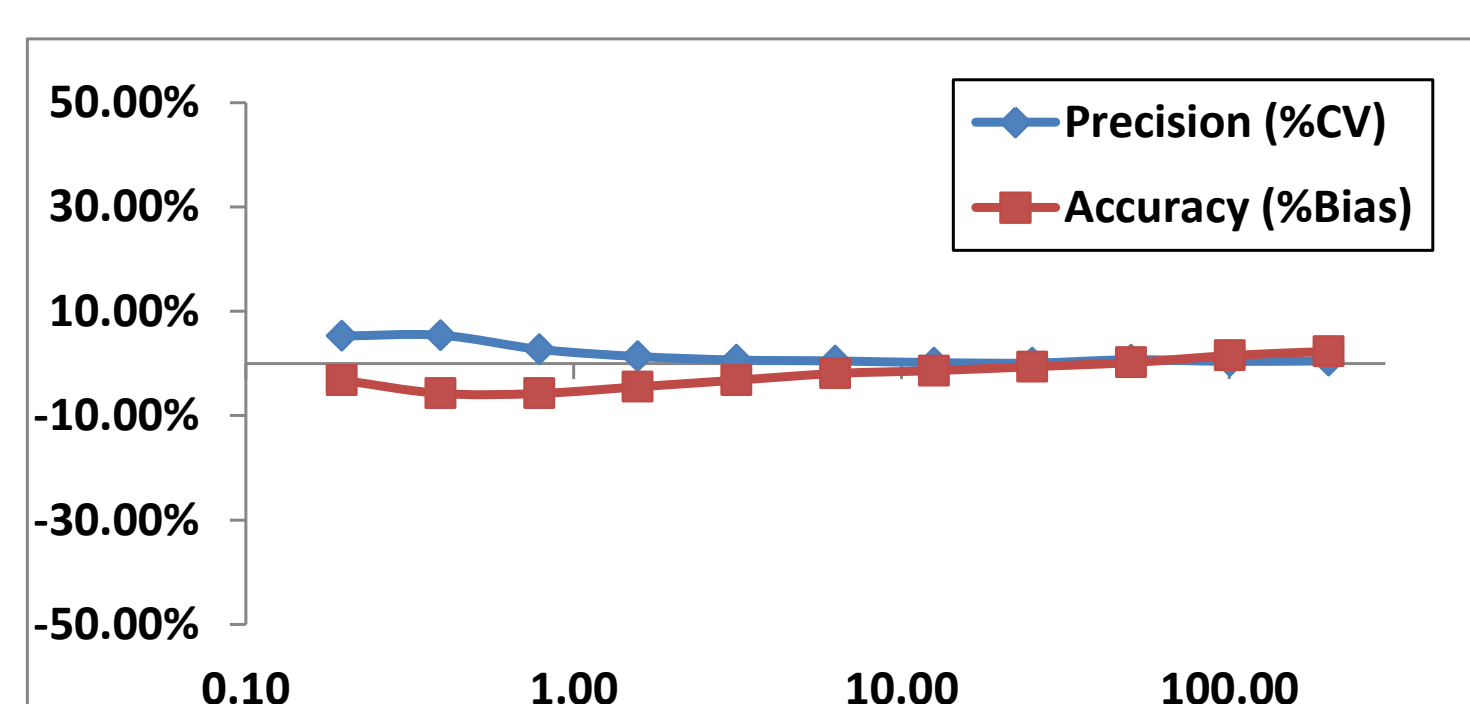
A stock solution of BSA exceeding 200 OD (10 mm path) was prepared dissolving BSA powder (Sigma: A4503-50g) in PBS. Gravimetric dilutions of the stock solution in PBS were prepared in order to evaluate a large concentration range (220 – 0.1 OD at 280nm, see figure below). Each BSA sample was measured in quadruplicate using 2 µl samples on the DropSense96 using DropPlates D+ and compared to results obtained with the Nanodrop 2000 spectrophotometer (Thermo). All data were normalized to a 10 mm path length and are shown in the graphs below. The data show a high linearity as the calculated coefficient of determination (R²) is 1.0003.



Samples	DropSense96				Nanodrop-2000		
Gravimetric OD	Average A280	STDEV A280	%CV A280	Average A280	STDEV A280	%CV A280	
BSA 1 220	218.4938	1.4387	0.70%	145.41	2.308	1.60%	
BSA 4 158.816	159.7886	0.7637	0.50%	152.45	1.975	1.30%	
BSA 5 138.326	138.7473	0.9271	0.70%	133.44	1.954	1.50%	
BSA 7 97.923	97.7698	0.5988	0.60%	94.85	1.245	1.30%	
BSA 9 47.998	47.8011	0.2340	0.50%	45.90	0.318	0.70%	
BSA 11 13.734	13.8468	0.0179	0.10%	13.51	0.16	1.20%	
BSA 13 4.437	4.3983	0.0113	0.30%	4.38	0.033	0.80%	
BSA 16 1.347	1.3050	0.0118	0.90%	1.32	0.018	1.40%	
BSA 17 0.885	0.8754	0.0154	1.80%	0.89	0.01	1.10%	
BSA 20 0.355	0.3328	0.0156	4.70%	0.34	0.007	2.10%	
BSA 21 0.179	0.1601	0.0065	4.10%	0.16	0.009	5.70%	
BSA 22 0.091	0.0761	0.0084	11.00%	0.10	0.057	55.80%	

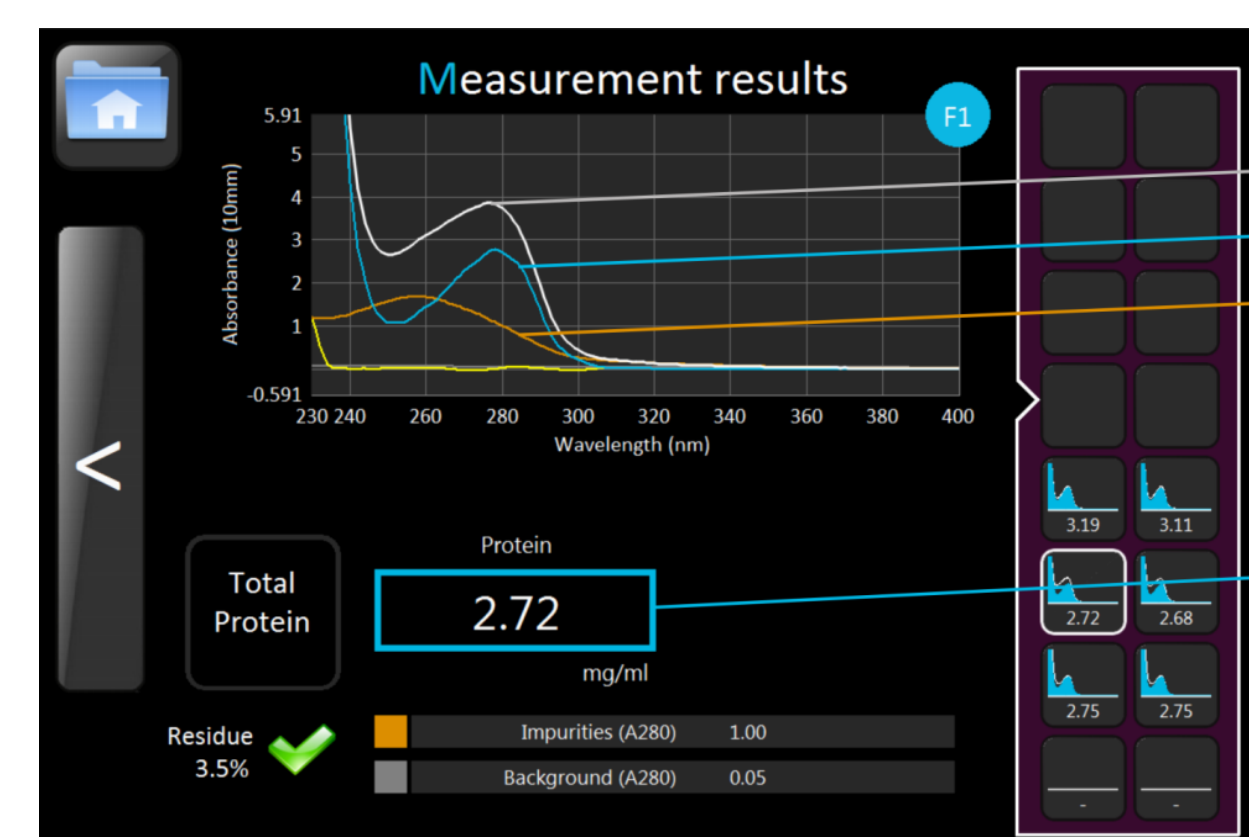
2. Accuracy of A280 protein quantification

The accuracy of the DropSense96 was evaluated by measuring a certified BSA solution in 150 mM NaCl, 0.1% NaN₃ (Sigma-Aldrich, Product nr P5369, Lot nr 101M6015). The certified stock concentration of 201 mg/ml BSA (by Biuret method) was used in a gravimetric 1/2 dilution series in the stock buffer as shown. Each dilution was measured 8 times on a DropSense96 using DropPlates D+ and the precision (%CV=100x [standard deviation/average]) and accuracy (%bias=100x [measured conc-actual conc]/actual conc) were determined.



Samples	DropSense96		
	Gravimetric conc (mg/ml)	Average conc (mg/ml, n=8)	STDEV (mg/ml)
BSA stock	201.00	205.66	1.03
BSA 1/2	100.50	102.02	0.41
BSA 1/4	50.25	50.34	0.36
BSA 1/8	25.13	24.97	0.02
BSA 1/16	12.56	12.39	0.02
BSA 1/32	6.28	6.16	0.03
BSA 1/64	3.14	3.04	0.02
BSA 1/128	1.57	1.50	0.02
BSA 1/256	0.79	0.74	0.02
BSA 1/512	0.39	0.37	0.02
BSA 1/1024	0.20	0.19	0.01

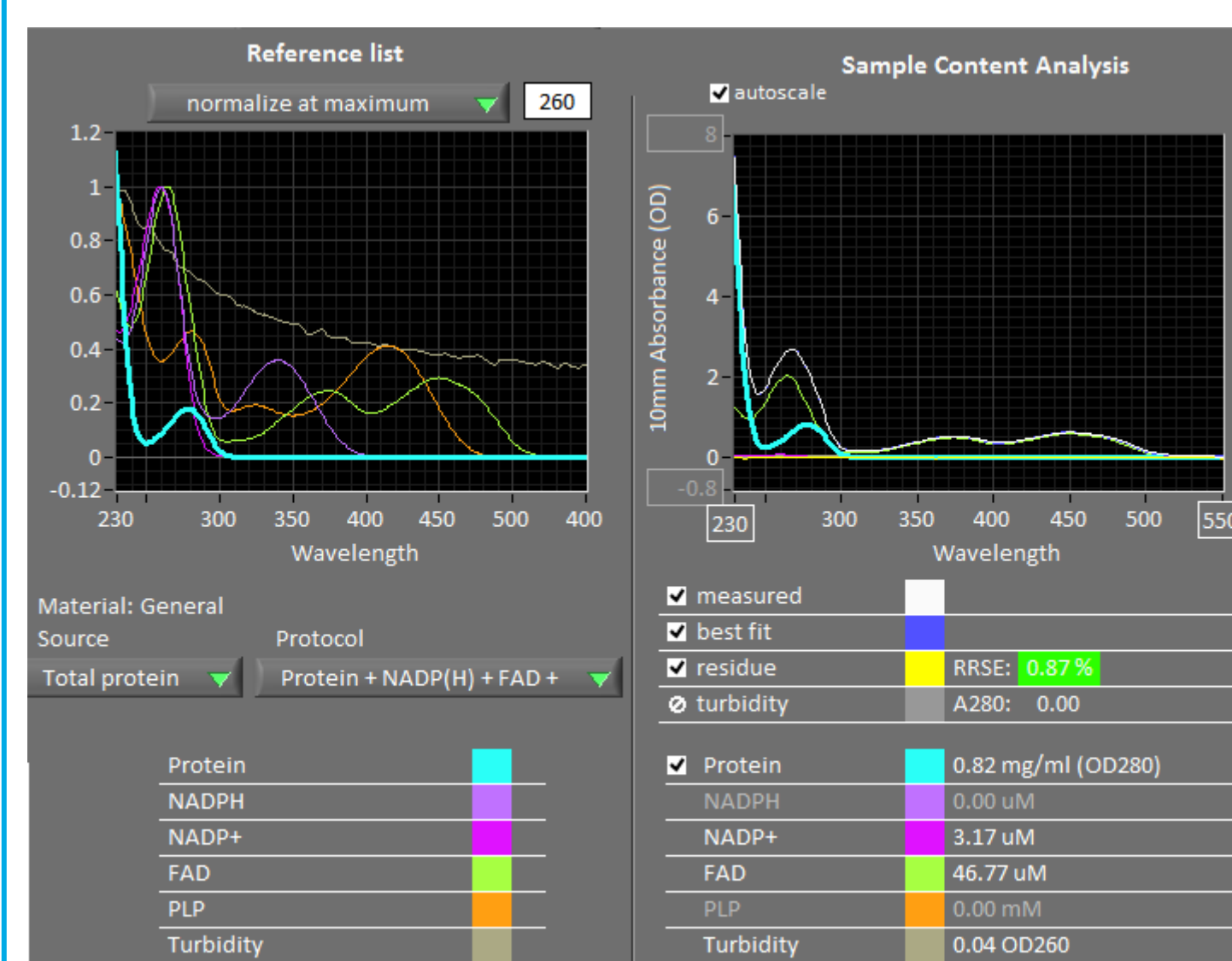
cDrop analysis



Principle
Measured UV/VIS spectrum
↓
Total Protein profile
Impurities profile
↓
Protein concentration =
A280 of blue profile * given E1%

1. Protein quantification in the presence of co-factors

As proof of principle, a set of samples was collected by a collaborative protein discovery lab including purified protein, purified co-factors FAD, NADP and NADPH and samples containing the protein and one or more co-factors. Triplicate measurements were performed on a DropSense96 using DropPlate S and analyzed using A280 and Trinean's proprietary cDrop software. The data illustrate the ability of the cDrop software to discriminate between the total protein profile and various co-factors within the same sample.



Sample name	A280 Conc (mg/ml)	cDrop Protein (mg/ml)	cDrop NADPH (µM)	cDrop NADP+ (µM)	cDrop FAD (µM)
blank	0.02	-	-	-	-
blank	0.03	-	-	-	-
blank	-0.03	-	-	-	-
NADP (0.5mM)	2.98	0	0	492.14	0
NADP (0.5mM)	2.98	0	0	492.47	0
NADP (0.5mM)	3.01	0	1.45	498.82	0.98
NADPH (0.5mM)	3.3	0	501.52	0	0
NADPH (0.5mM)	3.31	0	500.86	0	0
NADPH (0.5mM)	3.32	0	496.62	0	0
FAD (0.05mM)	1.24	0	0.1	0	50.05
FAD (0.05mM)	1.21	0	0	0	50.65
FAD (0.05mM)	1.13	0	0.29	0.04	46.43
protein (1mg/ml)	1.02	0.94	4.24	1.63	0
protein (1mg/ml)	1.05	0.98	3.9	2.29	0
protein (1mg/ml)	1.07	1.01	3.08	0.49	0
protein/NADP/NADPH	2.39	0.68	145.56	121.63	0
protein/NADP/NADPH	2.4	0.67	145.94	122.57	0
protein/NADP/NADPH	2.4	0.68	144.29	121.36	0
protein/FAD	1.99	0.82	0	3.17	46.77
protein/FAD	1.95	0.82	0	2.05	46.08
protein/FAD	1.99	0.83	0	2.33	45.71

2. Protein quantification in crude cell lysates

An evaluation was done in collaboration with a protein R&D lab where mammalian cell cultures were lysed using reporter lysis buffer (Promega) and loaded directly onto DropPlates S for quantification. The results below illustrate that the cDrop software is able to discriminate interfering nucleic acids and buffer chemicals from the total protein content within the measured UV/VIS spectrum. For this particular example, cDrop results show a high correlation with the BCA protein assay used in the lab as standard quantification tool, even when samples were spiked with more nucleic acids (Lysate 4 spiked with 100 ng/µl and 200 ng/µl DNA).

